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PRELIMINARY OBSERVATIONS OF LUNG INJURY PRODUCED BY INSTILLATION OF HF IN ACIDIC AND NEUTRAL BUFFER

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Abstract

Perfluoroisobutylene (PFIB) is an extremely toxic organofluoride that can be produced during pyrolysis of tetrafluoroethylene polymers, including Teflon®. Inhalation of PFIB at very low concentrations causes acute lung injury, the hallmark of which is pulmonary edema. Several lines of evidence have suggested that hydrolysis of PFIB and resulting production of hydrofluoric acid may be responsible for pulmonary damage. In order to investigate the potential involvement of hydrofluoric acid in producing lung injury and its relationship to the mechanism of fluorocarbon toxicity, we have compared the pulmonary injury produced by PFIB, by dissociated (H^+ and F^-), and by undissociated (HF) hydrofluoric acid in the deep lung. By delivering hydrofluoric acid by intratracheal instillation in neutral buffer, we demonstrate that F^- produces no significant pulmonary injury as assessed by increases in lung weight and ultrastructural changes. Similarly, instillation of acid buffer alone demonstrated that H^+ did not produce detectable lung injury. Instillation of HF produced changes in lung weight and ultrastructure similar to those observed in PFIB-treated rats. However, the ultrastructural studies show that in contrast to inhalation of PFIB, which produces both endothelial and epithelial cell damage, instillation of HF appears to exert its injurious effects only upon epithelial cells.

Introduction

Perfluoroisobutylene (PFIB) is one of a number of volatile fluoroalkenes that cause severe lung injury when inhaled at low concentrations (1). The hallmark feature of the injury is a breach in the permeability of the lung's air-blood barrier, with ensuing pulmonary edema (2,3). A variety of chemical mechanisms have been proposed to explain the toxic effects of this important class of pulmonary irritants, but there is no consensus as to the actual mechanism(s) involved. Because many toxic fluorocarbons are readily susceptible to hydrolysis under certain conditions, and because hydrofluoric acid and these toxic fluorocarbons produce similar lung injury, hydrolysis and production of hydrofluoric acid have been suspected to be involved in producing pulmonary injury (4-6). On the other hand, pulmonary injury caused by hydrofluoric acid exposure is primarily restricted to the upper respiratory tract, whereas PFIB and related fluorocarbons are known to injure the deep lung. Also, the relative toxicity of hydrofluoric acid

(>1200 ppm for 10 min) is much less than PFIB (18 ppm for 10 min). The marked differences in relative toxicities and sites of injury suggest that hydrofluoric acid and PFIB operate by different mechanisms.

Arguments based on the differences in relative toxicities and sites of injury are confounded by possible differences in the hydrophilic/hydrophobic nature of hydrofluoric acid and PFIB and the resulting differences in sites of absorption in the airways. Because hydrofluoric acid is hydrophilic, it may be efficiently absorbed in the moist upper airways and thereby produce injury in the upper respiratory tract while sparing the more peripheral deep lung. Based on estimates of airway surface area and measurements of absorption of hydrofluoric acid in the nasal region of the rat, we have recently estimated that essentially all hydrofluoric acid breathed via the oral airway could be removed by the time the inhaled airstream reached 9-10 generations of the rat's tracheobronchial tree (7).

In order to determine if hydrofluoric acid, when delivered to the deep lung, can produce similar injury to PFIB, we have used the instillation of hydrofluoric acid in buffered saline as a model to introduce hydrofluoric acid into the lung's alveoler region. The extent of lung injury produced by hydrofluoric acid was compared to that produced by inhaled PFIB using lung gravimetric and ultrastructural criteria. In addition, we have compared the extent of lung injury produced by undissociated HF (8), and by the products of dissociation H^+ and F^-, in order to assess which of these potential species generated by hydrofluoric acid is most likely involved in producing lung injury.

Experimental Design

The experiments were designed to compare the pulmonary injury produced by four different doses of hydrofluoric acid in buffered saline with that produced by inhalation of PFIB. In addition, we compared the pulmonary injury produced by hydrofluoric acid in acid buffered saline (where the hydrofluoric acid is present primarily as HF) with that in neutral buffered saline (where the hydrofluoric acid is present primarily as F^-) given by intratracheal instillation. Because the pK_a for hydrofluoric acid is 3.19, we selected conditions of pH=2.1 in phosphate-buffered saline (PBS) to give >90% HF ($[HF]/[F^-] = K_a/[H^+]$), and pH=7.4 to give >99% F^- ($[F^-]/[HF] = [H^+]/K_a$). The hydrofluoric acid in PBS was delivered to the lungs of rats by instillation of 0.5 ml of 0.22 mM, 2.2 mM, 22 mM and 44 mM hydrofluoric acid solutions resulting in dose quantities of 0.11μM, 1.1μM, 11μM and 22μM, respectively. Instillations of PBS, pH=7.4 and PBS, pH=2.1, were also included in the experimental design as controls for the injury produced by instillation of neutral- and acid-buffered saline alone. Pulmonary injury was assessed by increases in lung wet weight (LWW) and right cranial lobe dry weight (RCLDW) 24 hours after exposure and by changes in the ultrastructure of lung sections fixed 3 hours after exposure. We note that preliminary studies have demonstrated that the intratracheal instillation of normal PBS causes no detectable evidence of lung injury, using lung gravimetric measurements and histology as end points. For purposes of comparison, we estimated the dose of PFIB received from inhalation of 100 mg/M^3 for 10 minutes, assuming an average minute ventilation of 220 ml and 100% deposition of the fluorocarbon. These assumptions give an estimated dose received from inhaled PFIB of 1.1 μM.

The intratracheal instillation of PBS solutions was performed with male Fischer 344 rats (SPF, 245-270 g) under Ethrane® anesthesia. Each group of rats consisted of 3-4 animals. Exposure to PFIB was performed as previously described (7). The animals were sacrificed 24 hr

after the instillations or PFIB exposures and their lungs were excised. The procedures for obtaining lung wet weights (LWW), and right cranial lobe dry weights (RCLDW) and electron micrographs have been described previously (7).

Results and Discussion

Lung wet weights and right cranial lobe dry weights determined 24 hours after exposure are shown in Figure 1.

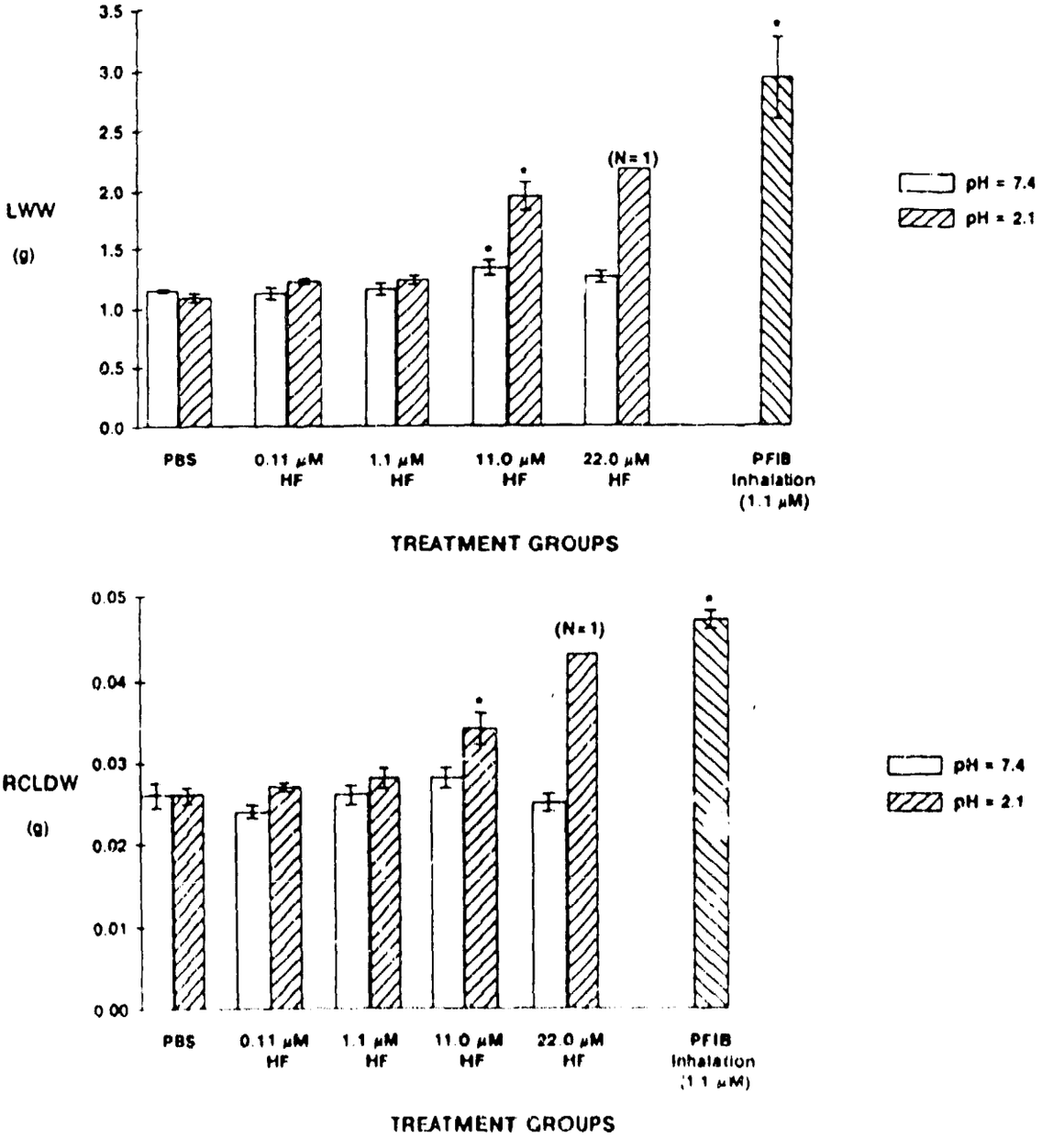

Figure 1: Lung wt weight (LWW) and right cranial lobe dry weight (RCLDW) 24 hours after exposure. * = significantly higher than PBS instilled controls, $p \leq 0.05$.

No significant increases in LWW or RCLDW were observed for rats exposed to either acidic PBS (pH 2.1), neutral PBS (pH 7.4), or for rats exposed to 0.11 μM, and 1.1 μM hydrofluoric acid in either acidic or neutral PBS. The ~7% increase in LWW observed for 11 μM hydrofluoric delivered in neutral (pH 7.4) PBS was significant at the p=0.05 level. However, based on the absence of any significance increase in LWW for 22 μM hydrofluoric acid in neutral PBS or increases in RCLDW for 11 μM and 22 μM hydrofluoric acid in neutral PBS, we suspect the statistical significance estimated for 11 μM hydrofluoric acid in neutral PBS is a type I statistical error. Statistically significant increases in LWW and RCLDW were observed for rats receiving 11 μM and 22 μM doses in acid-buffered (pH 2.1) PBS. In the group of 3 rats exposed to 22 μM HF in acid-buffered PBS, the instillation of one rat was unsuccessful, and one rat died within 12 hours after exposure; consequently, we obtained gravimetric data for only one rat at this dose.

Because hydrofluoric acid is a weak acid in aqueous solution, the hydrofluoric acid in acid-buffered PBS exists primarily in the undissociated form HF; in neutral-buffered PBS, the hydrofluoric acid exists primarily as F^-. This fact and the lung gravimetric data presented above demonstrate that at 11 and 22 μM doses given by instillation, HF produces more lung injury than F^-. This observation suggests that HF rather than F^- is the agent responsible for injury in pulmonary tissue exposed to hydrofluoric acid. The observations that neither neutral PBS or acidic PBS alone produced significant lung injury shows that neither the instillation procedure or the delivery of acid (H^+) in PBS results in significant tissue injury. These data argue for the involvement of HF rather than F^- or H^+ as the active species in pulmonary injury produced by exposure to hydrofluoric acid.

The similar increases in LWW and RCLDW observed for PFIB and HF suggest that these agents may act through the same mechanism, implying that hydrolysis of PFIB and production of HF is important in the mechanism of PFIB-induced lung injury. On the other hand, the injury produced by 11 and 22 μM doses of HF is less than that produced by inhalation of PFIB at 100 mg/M^3 for ten minutes, even though the dose of HF was 10 and 20 times greater than that estimated for PFIB. This observation suggests that PFIB is many times more effective at producing lung injury and that HF and PFIB may act by different mechanisms. However, direct comparisons of the toxic effects of PFIB given by inhalation and HF given by instillation are complicated by the dissimilar delivery mechanisms. Part of the differences in dose-response may be explained by the fact that the intratracheal instillation procedure results in uneven distribution of instilled material in the lung with some regions receiving the bulk of the material, while other regions may receive no or little material. Studies performed in our laboratory on the distribution of particles in rat lung following intratracheal instillation suggest that only about 30-40% of the peripheral alveoli receive the test material. With a gaseous material like PFIB, on the other hand, deposition in the alveolar region may be substantially more homogeneous, and the response to the material may be greater. In addition, the observed differences in dose-response for HF and PFIB may reflect differences in the intracellular and/or intercellular distribution of the agents. We believe an important property of PFIB and other fluorocarbons is their lipophilic character, which could result in relatively facile penetration into and through cell membranes. This view is explored further in the discussion of the ultrastructure results below.

The ultrastructural changes that occur in the lung as of 1 and 3 hrs after the inhalation of 100 mg/M^3 PFIB for 10 min have been reported elsewhere (2). Briefly, the earliest (1 hr post exposure) detectable evidence of injury is alveolar epithelial and endothelial cell blebbing. This

outcome was accompanied by an abnormal increase in blood monocytes and polymorphonuclear leukocytes in the pulmonary capillaries. As of 3 hr after exposure, alveolar epithelial cell blebbing progressed to cell herniation, cell necrosis, and cell exfoliation. Epithelial target cells included both the type I and type II pneumocytes. In some instances, extensively swollen endothelial cells with relatively rarified appeared to occupy significant fractions of capillary lumens, and fenestrations in the endothelial barrier were occasionally observed so that vascular constituents were given direct access to the interstitial region. Two cell types that appeared to be relatively resistent to the toxic effects of PFIB were the alveolar macrophages and interstitial lung fibroblasts, although the former type of cells often times were observed to be phagocytizing fibrin and lamellar material abnormally present in the alveolar space compartment.

Unlike the above observations, no significant evidence of ultrastructural injury in the alveolar region was observed following the intratracheal instillation of PBS at a pH of 7.4 or 2.1 (micrographs not shown). As well, the parenchymal region of the lung did not appear to be significantly affected by the instillation of as much as 22 μM hydrofluoric acid when delivered in PBS at a pH of 7.4, Figure 2A. On the other hand, substantial evidence of lung injury was observed when the hydrofluoric acid was administered in PBS at a pH of 2.1, Figure 2B. Hallmark features of such injury included the destruction and exfoliation of type I pneumocytes, and the swelling and exfoliation of type II pneumocytes. The appearance of fibrin, amorphous proteinaceous material, and lamellar material in the alveoli were also commonly observed.

Overall, the major ultrastructural difference between injury induced by hydrofluoric acid administered at a low pH and the injurious response induced by the inhalation of PFIB is that the PFIB caused pronounced damage to both the alveolar epithelial and endothelial cells whereas the hydrofluoric acid appeared to target only the epithelial cells lining the alveolar surface.

Electron Micrograph Figure Legends

Figure 2A: Electron micrograph of the alveolar region of a lung that was instilled with 22 mm hydrofluoric acid in PBS, pH 7.4. No ultrastructural evidence of damage to the type I epithelial cells (arrows) is apparent. The endothelial linings of the pulmonary capillaries are normal in appearance, and no abnormal material, e.g., fibrin, proteinaceous or lamellar material, is present in the alveolar spaces (ALV). A type II pneumoncyte (II) on an alveolar surface and a fibroblast (F) in the alveolar interstitial region show no ultrastructural evidence of injury.

Figure 2B: Electron micrograph of the alveolar region of a lung that was instilled with 11 mm hydrofluoric acid in PBS, pH 2.1. Type I alveolar epithelial cells show extensive destruction (cytoplasmic rarification and lysis), and many of these epithelial cells or their remnants have lifted off the alveolar surfaces (arrows). An apparently exfoliated type II pneumocyte (II) is also present in an alveolus. Aside from cutting artifacts, the endothelial cells lining the pulmonary capillaries are normal in appearance.

Figure 2C: Electron micrograph of the alveolar region of a lung that was instilled with 22 mm hydroflouric acid in PBS, pH 2.1. Type I epithelial cells show extensive cytoplasmic rarification, lysis, and detachment (small arrows). A type II pneumocyte is swollen and the cytoplasm of this cell shows extensive internal blebbing (open arrow). Cell debris and amorphous proteinaceous material are present in the alveolar spaces (ALV).

Conclusions

The lung gravimetric and ultrastructural results presented here generally support the involvement of PFIB hydrolysis and the production of HF as the primary mechanism of PFIB-induced lung injury. We speculate that the dose-response differences observed between PFIB and HF reflect differences in the delivery and distribution of the toxic agents in the deep lung. The ultrastructural data demonstrate that the lung injury produced by instillation of HF is limited primarily to the epithelial side of the lung air-blood barrier, in contrast to PFIB, which damages cell types at both endothelial and epithelial surfaces. This difference in sites of cellular injury within the lung tissue may be the result of the more hydrophilic nature of HF, which may limit its transport through cell membranes relative to the more hydrophobic PFIB.

Acknowledgments

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8. To avoid possible confusion in discussing the effects of undissociated or dissociated hydrofluoric acid we will use the convention that the full name hydrofluoric acid will be used when the state of dissociation is unknown or when we wish to include both dissociated and undissociated forms and that the symbol HF will be used exclusively to denote undissociated hydrofluoric acid. In addition, we will use the symbol H^+ to refer to the hydrated proton in aqueous solution.

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